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Washing and Sanitizing Raw Materials for Minimally Processed Fruit and Vegetable Products*

Gerald M. Sapers

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INTRODUCTION

Guidelines for packing fresh or minimally processed fruits and vegetables generally specify a washing or sanitizing step to remove dirt, pesticide residues, and microorganisms responsible for quality loss and decay. Additionally, this step is used to precool cut produce and remove cell exudates that adhere to product cut surfaces and may support microbial growth or result in discoloration. The U.S. Food and Drug Administration's *Guide* calls for removing "as much dirt and mud as practicable from the produce before it leaves the field." The produce should be cleaned (washed and rinsed with processing water of such quality that it does not contaminate the produce) to be visually free of dust, dirt, and other debris and sanitized (treated "by a process that is effective in destroying or substantially reducing the numbers of microorganisms of public health concern, as well as other undesirable microorganisms, without adversely affecting the quality of the product or its safety for the consumer") (FDA, 1998).

In recent years, increasing attention has been focused on the microbiological safety of fruits and vegetables and, in particular, on interventions to kill or remove human pathogens from fresh produce. The number of outbreaks of food-borne illness associated with fresh produce appears to be increasing (Tauxe et al., 1997; NACMCF, 1999; DeWaal et al., 2000). In the U.S., the majority of reported cases involved a limited number of commodities: alfalfa sprouts, lettuce or other salad greens, melons, unpasteurized juices including fresh apple and orange juices, tomatoes, berries, cabbage, and unspecified fruits (NACMCF, 1999; DeWaal et al., 2000). Some of these have been minimally processed products (Francis et al., 1999). The causative organisms, where identified, included *Escherichia coli* O157:H7, *Salmonella* species, *Shigella* species, *Cyclospora cayetanensis*, Hepatitis A virus, Norwalk-like virus, and, rarely, *Listeria monocytogenes* and *Clostridium botulinum* (Beuchat, 1996; NACMCF, 1999). A key goal of washing and sanitizing fresh or minimally

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processed fruits and vegetables, therefore, is the removal or inactivation of pathogenic microorganisms.

Until recently, relatively little information was published concerning the efficacy of washing and sanitizing treatments in reducing microbial populations on produce. The limited data published suggested that conventional washing and sanitizing methods, even using some of the newer sanitizing agents such as chlorine dioxide, ozone, and peroxyacetic acid, were not capable of reducing microbial populations by more than 90 or 99% (Beuchat, 1998; Brackett, 1999). Although such reductions represent a large decrease in the numbers of microorganisms present on the commodity and may result in significant improvements in product quality and shelf-life, they are not equivalent to surface pasteurization and may be inadequate to ensure product safety. Thus, there is a need to examine the factors that limit the efficacy of washing in reducing microbial populations on produce and to devise means of overcoming such limitations. New washing and sanitizing treatments must not only be effective, but they also must be compatible with commercial packing and processing practices and technical capabilities. New treatments must be affordable and safe to carry out, have no adverse effect on quality, and be approved by applicable regulatory agencies. Acceptance by industry also may depend on regulatory constraints regarding use of the term "fresh," conformance to organic food product labeling requirements, and perceived consumer attitudes regarding irradiation and "chemicals."

The problem of decontaminating produce by washing or application of chemical sanitizing treatments cannot be understood in isolation; we must take into account not only the size of the microbial load on fruits and vegetables but also the way in which microorganisms attach and survive on produce surfaces. The condition of the attached microflora will depend, in many cases, on how and when the produce became contaminated. Generally, it is more difficult to decontaminate produce than it is to avoid contamination. Therefore, pre- and postharvest interventions that reduce the risk of contamination will make the job of sanitizing produce easier.

In this chapter, the focus will be on bacterial contamination of produce, especially contamination with human pathogens. Major factors that limit the efficacy of conventional washing methods and their relationship to the circumstances of contamination will be examined. The ability of conventional washing and sanitizing agents and produce washing equipment to reduce microbial loads will be reviewed. Finally, new developments in washing and sanitizing technology and the prospects for significant improvements in decontamination efficacy will be discussed.

Nonthermal physical treatments such as exposure to ionizing radiation, high intensity pulsed light, and high pressure have the capability to sterilize or pasteurize some commodities and may have some application to minimally processed fruit and vegetable products. However, since these processes are substantially different from washing and sanitizing treatments, they will not be discussed in this chapter. Similarly, a number of natural products have antimicrobial activity. However, in most cases, these agents do not exert their effects within the time frame of a wash but act as preservatives, inactivating microorganisms or suppressing their growth during storage. Such applications also lie outside the scope of this chapter.

FACTORS LIMITING THE EFFICACY OF WASHING

SOURCES OF MICROBIAL CONTAMINATION

Preharvest Contamination

Ultimately, human pathogens associated with produce can be traced to human or animal fecal contamination. Produce is grown on farms, not in clean rooms, and it is not possible to exclude animals completely from the fields and orchards where produce originates. Human pathogens associated with food-borne illness have been found in cattle (Faith et al., 1996), sheep (Kudva et al., 1996), deer (Rice et al., 1995), wild birds (Wallace et al., 1997), and amphibians (Parish, 1997). They might be carried by insects (Janisiewicz et al., 1999a; Iwasa et al., 1999; Kobayashi et al., 1999). Outbreaks of *E. coli* O157:H7 associated with apple cider have been attributed to cattle grazing in apple orchards (Besser et al., 1993). Contamination of produce might result from fertilizing with improperly composted manure in which human pathogens survive. Low-growing fruits and vegetables might be splashed with contaminated muddy water during a heavy rainstorm. The following examples, taken from recent field studies carried out by scientists at the USDA's Eastern Regional Research Center, illustrate the contamination problem.

Alfalfa sprouts have a history of involvement in outbreaks of illness caused by *Salmonella* and occasionally by *E. coli* O157:H7 (Taormina et al., 1999). In an investigation of the microbiological safety of alfalfa sprouts, an example was encountered of probable fecal contamination of alfalfa seeds, probably due to the practice of allowing cattle to graze in alfalfa fields after the last harvest of the growing season. Seed and debris fractions (weed seeds, plant fragments, insect parts, and soil) obtained from commercial seed cleaning equipment were found to be positive for generic *E. coli* and confirmed *Salmonella* (serotypes Bredeney and Worthington). Contamination apparently occurred when these organisms survived the winter in cattle feces deposited in the field and became attached to the new crop during the following spring (Fett and Sapers, 1997).

Water used for irrigation or to make up pesticide or other sprays might represent another potential source of fecal contamination if it is obtained from a contaminated pond, stream, or irrigation canal where animals drink or where run-off from adjacent fields or pastures occurs. Generic *E. coli* was detected in each of these water sources at orchard locations associated with *E. coli* contamination of apples intended for cider production (Riordan et al., 2001).

Produce might be contaminated by windblown dust from a nearby pasture or feedlot that contains particles of desiccated cattle feces and human pathogens. Evidence of this scenario was seen in apples obtained from an orchard located downwind of a feedlot (Fett and Sapers, 1997). Soil from the orchard tested positive for coliforms and generic *E. coli*. The apples were visibly dirty in the stem cavity area, and many apples tested positive for coliforms and generic *E. coli*.

Contamination from microorganisms in dust or irrigation water could complicate the problem of decontamination by favoring microbial attachment in relatively inaccessible sites such as upward facing calyx or stem areas of apples where dust and water can be trapped. Microbial penetration and growth within punctures result-

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ing from bird pecks or hail damage are possible consequences of such contamination. The moist environment and nutrient availability prevailing in such attachment sites also might favor biofilm formation, which would increase microbial resistance to washing and sanitizing agents.

Growers should make every effort to exclude animals or their feces from their fields and orchards by use of suitable fencing or bird-repellent devices, by avoiding exposure to contaminated dust and irrigation sources, and by following other pertinent good agricultural practices and guidelines (See Interventions section below).

Postharvest Contamination

Produce might become contaminated with human pathogens as a result of contact with contaminated soil or water during harvesting, postharvest handling, or processing. Outbreaks of food-borne illness have been associated with production of unpasteurized cider from apples that had fallen on the ground (Besser et al., 1993; CDC, 1997). Bins or other containers used to hold harvested produce might be contaminated with soil, decayed fruit or vegetable fragments, or other debris. Deer, which are known sources of *E. coli* O157:H7 (Rice et al., 1995), are frequently found in orchards near wooded areas and might defecate on the ground beneath the trees where bins were stored. Stacking such bins might allow potentially contaminated dirt adhering to the bottom of one bin to fall into the bin beneath. Storing loaded bins under shade trees in the field would increase the chance of produce contamination by bird droppings. Trucks used to ship produce from the grower to the packer or processor might be contaminated with human pathogens, especially if the trucks were used previously to transport animals such as pigs or poultry and were not adequately cleaned and sanitized. Water used in drenchers, hydrocoolers, dump tanks, or flumes might be contaminated if obtained from a contaminated source. If such water were recycled without further treatment, opportunities for cross-contamination would exist. Cross-contamination also can take place in a packing or processing plant if equipment in contact with potentially contaminated produce is not regularly cleaned and sanitized.

Interventions to Avoid Postharvest Contamination

A number of interventions can be implemented to avoid postharvest contamination of produce with human pathogens. Various government agencies and industry associations have published comprehensive guidelines for the produce industry. A description of good manufacturing practices appears in the U.S. Code of Federal Regulations (21CFR 110, 1994a). The Food and Drug Administration has issued a *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables* (FDA, 1998). The International Fresh-cut Produce Association (IFPA) has published *Food Safety Guidelines for the Fresh-cut Produce Industry* (Zagory and Hurst, 1996). This document calls for chlorination of water used for field washing and in packing houses and provides information about the characteristics of various sanitizing agents added to fresh-cut processing water or used to sanitize plant equipment. The Codex Committee on Food Hygiene has developed a *Code of Hygienic Practice* that is now in draft form (Couture, 1999). The Canadian government has published a *Code of*

Practice for production of unpasteurized fruit juices (Canadian Food Inspection Agency, 1998).

Implementation of a hazard analysis critical control point (HACCP) plan by processors and HACCP-like plans (e.g., good agricultural practices [GAPs] and good manufacturing practices [GMPs]) by packers can result in exclusion of contaminated raw materials and implementation of effective decontamination treatments. Such plans are described in the IFPA guidelines (Zagory and Hurst, 1996), a report of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1997), and regulations promulgated by the FDA (FDA, 2001).

Cleaning and sanitizing represent key interventions in any program to avoid human pathogen contamination of fresh or minimally processed produce. Antimicrobial agents such as chlorine (or hypochlorous acid obtained from sodium or calcium hypochlorite or compressed chlorine gas), chlorine dioxide, ozone, or peroxyacetic acid can be added to process water used in dump tanks, flumes, and washers to reduce the bacterial load and levels of any human pathogens that might be present. This is an effective means of minimizing cross-contamination, i.e., the attachment of human pathogens suspended in the process water to uncontaminated produce passing through the water. However, as discussed above, the ability of these antimicrobial agents to kill bacteria already attached to the surface of fruits and vegetables is limited to population reductions of 90 to 99%.

Customarily, population reductions resulting from antimicrobial treatments are expressed as log reduction values rather than as percentages, to avoid the need to work with very large numbers. Log reductions are calculated by subtracting the logarithm of the microbial population surviving a wash or sanitizer treatment ($\log_{10}\text{CFU/g}$, where CFU is the number of colony forming units, i.e., the number of viable microorganisms recovered and enumerated on specific microbiological growth media) from the logarithm of the initial microbial population before treatment. Log reductions of 1, 2, 3, 4, and 5 are equivalent to percentage reductions of 90, 99, 99.9, 99.99, and 99.999, respectively. Henceforth in this chapter, log reductions will be used in place of percent reductions.

The FDA has established a 5-log reduction in the human pathogen population in fresh apple cider as a goal for pasteurization treatments (flash pasteurization, UV pasteurization) or other interventions that could be applied to the juice (FDA, 2001). A 5-log reduction in human pathogens on seeds for sprout production has been proposed by the National Advisory Committee on Microbiological Criteria for Foods but is not required by the FDA (Anonymous, 1999b; FDA, 1999). Although the 5-log reduction target might not be directly applicable to or required of minimally processed (i.e., fresh-cut) fruit and vegetable products, it does indicate the kind of population reduction goal that food safety authorities visualize for high-risk products.

Equipment used in conveying, sorting, cutting, peeling, or performing other operations on fresh produce might be sources of human pathogens if fragments of contaminated fruits and vegetables become lodged within the equipment so that microbial growth can occur or if biofilms containing human pathogens become established on food contact surfaces. When incoming produce passes through the packing or processing line, cross-contamination can occur as fragments detach and contact fruit or vegetable surfaces. To avoid this situation, equipment must be cleaned

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TABLE 11.1
Annual Buyers' Guides as Sources for Washing and Sanitizing Agents and Equipment

Category	Food Quality Buyer's Guide ^a	Food Processing Guide and Directory ^b	IFPA Membership Directory and Buyer's Guide ^c	Prepared Foods Food Industry Sourcebook ^d
Surfactants and detergents	X	X		X
Sanitizers	X	X	X	X
Chlorine	X	X		
Ozone		X		
Chlorine dioxide		X	X	
Produce washing equipment		X	X	
Sanitizing equipment	X	X	X	X
Cleaning equipment	X	X	X	X
Sanitation, GMP, and HACCP consultants	X	X	X	X
Contract laboratory services	X	X	X	X
Microbiological supplies and test kits	X	X	X	X

^a Supplement to *Food Quality Magazine*, Carpe Diem Communications, Inc., Yardley, PA. 1999/2000. Online guide at www.foodquality.com.

^b Supplement to *Food Processing*, Putnam Publishing Co., Itasca, IL, October, 2000. Online guide at www.foodprocessing.com.

^c Published by International Fresh-cut Produce Association, Alexandria, VA. 2000/2001. See www.fresh-cuts.org.

^d Supplement to *Prepared Foods*, Des Plaines, IL, 1996. Online guide at www.preparedfoods.com.

frequently with suitable detergents to remove produce fragments and biofilms and then sanitized to kill microbial contaminants. Approved sanitizers suitable for equipment and food contact surfaces include chlorine-based sanitizers, iodine-based sanitizers, quaternary ammonium compounds, and acid-anionic sanitizers (Zagory and Hurst, 1996; 21CFR178.1010, 2000). Sources of these products can be located in directories of suppliers to the food industry (Table 11.1).

MICROBIAL ATTACHMENT TO PRODUCE

The condition and location of microorganisms on produce surfaces affect their resistance to detachment by washing agents and to inactivation by antimicrobial agents. Microbial resistance to sanitizing washes will depend in part on whether the

TABLE 11.2
Effect of Interval between Inoculation of Apples with *E. Coli* (ATCC 25922) and Water Washing on Bacterial Population Reduction^a

Time after Inoculation (hr)	Log ₁₀ CFU/g ^b				Log ₁₀ CFU/g Reduction from Wash ^f	
	4°C		20°C		4°C	20°C
	Inoculated Control	After Wash	Inoculated Control	After Wash		
0.5	4.40 ^c	3.46 ^c	4.35 ^{c,d}	3.38 ^c	0.94*	0.97**
24	3.89 ^{c,d}	3.22 ^c	4.80 ^c	4.33 ^{c,d}	NS	0.47**
48	3.88 ^{c,d}	3.97 ^c	4.06 ^d	4.65 ^c	NS	-0.59**
72	3.66 ^d	3.64 ^c	4.18 ^{c,d}	3.88 ^{d,e}	NS	NS

^a From Sapers, G.M. et al., *J. Food Sci.*, 65:529–532, 2000a.

^b Mean of duplicate trials.

^{c,e} Within the same column, means with no letter in common are significantly different ($p < 0.05$) by Bonferroni LSD.

^f Significance of log₁₀CFU/g reduction tested by ANOVA: * ($p < 0.05$), ** ($p < 0.01$), NS = not significant.

microbial populations have become firmly attached, are concentrated in inaccessible sites, have penetrated into the interior of the commodity, or have become incorporated into biofilms. Each of these factors will affect the success of decontamination.

Rapidity of Attachment

One of the characteristics of bacterial attachment to fruits and vegetables is the rapidity of attachment to the commodity surface. The effectiveness of washing will depend on the time interval between contamination and washing. Data obtained with apples that were artificially inoculated with *E. coli* and then held for various times before washing with water indicate that an interval of 30 min between inoculation and washing resulted in a 1-log population reduction (Table 11.2). However, after 24 h, essentially all of the bacteria were firmly attached and could not be removed by washing (Sapers et al., 2000a). Similar results were obtained with cantaloupe artificially inoculated with a nonpathogenic *E. coli* or *Salmonella stanley* and then washed with water, 1000 ppm chlorine (as sodium hypochlorite), or 5% hydrogen peroxide at various intervals after inoculation (Ukuku et al., 2001; Ukuku and Sapers, 2001). Immediately after inoculation, the attached *E. coli* and *S. stanley* populations were detached and/or killed by washing with the chlorine or hydrogen peroxide solutions (but not by washing with water), resulting in reductions exceeding 3.5 to 4.5 log₁₀ CFU/cm². However, after 72 h, washing with these antimicrobial agents was much less effective in reducing the bacterial populations, resulting in reductions less than 3 log₁₀ CFU/cm² for *E. coli* and less than 2 log₁₀ CFU/cm² for *S. stanley*. Studies with pepper disks have shown that initial attachment of *Salmonella* to cut surfaces is very rapid and is directly related to the inoculum population but not to exposure time (0.5 to 16 min) (Liao and Cooke, 2001).

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TABLE 11.3
Distribution of *E. Coli* (ATCC 25922) on Surfaces of Inoculated Apples before and after Washing with 5% H₂O₂ at 50°C^a

Location	Log ₁₀ (CFU/cm ²) ^b			
	24 h after Inoculation		72 h after Inoculation	
	Inoculated	Washed ^c	Inoculated	Washed ^c
Skin on wedges	4.77 ^e	2.05 ^e	4.37 ^e	1.63 ^e
Skin at calyx end of core	7.26 ^e	5.20 ^d	6.79 ^d	4.46 ^d
Skin on stem end of core	6.63 ^d	5.06 ^d	5.61 ^d	4.89 ^d

^a From Sapers, G.M. et al., *J. Food Sci.*, 65:529–532, 2000a.

^b Based on calculated surface area of skin.

^c Washed 1 min in 5% H₂O₂ at 50°C.

^{d-e} Within the same column, means with no letter in common are significantly different ($p < 0.05$) by Bonferroni LSD.

Attachment in Inaccessible Sites

When bacteria attach to the surfaces of fruits and vegetables, they tend to concentrate where there are more binding sites. Attachment also might be in stomata (Seo and Frank, 1999), indentations, or other natural irregularities on the intact surface where bacteria could lodge. Bacteria also might attach at cut surfaces (Takeuchi et al., 2000; Liao and Cooke, 2001) or in punctures or cracks in the external surface (Burnett et al., 2000).

Data obtained with apples artificially inoculated with *E. coli* suggest greater attachment to skin in the calyx and stem areas than elsewhere on the apple (Table 11.3). When the inoculated apples were held 24 h and then washed with 5% hydrogen peroxide, many more survivors per square cm of skin surface were in the calyx and stem areas than elsewhere on the apple surface (Sapers et al., 2000a). Bacteria in the stem and calyx areas may adhere better to the irregular skin surface and to the flower parts of the calyx than to the smooth skin surface. Bacteria in these locations are inaccessible or at least less accessible to the washing agent, which requires physical contact to be effective. High levels of bacteria are found in the calyx and stem areas of naturally contaminated apples (Riordan et al., 2001).

Salmonella chester attach preferentially to the cut surfaces of apple and green pepper disks, where they survive washing to a much greater extent than bacteria on the intact apple and pepper surfaces (Liao and Sapers, 2000; Liao and Cooke, 2001). Since fresh-cut fruits and vegetables provide extensive cut surfaces for bacterial attachment, it is especially important with these products that exposure to human pathogens be avoided.

Attachment and Growth in Punctures

Some commodities such as certain apple and pear varieties, zucchini squash, potatoes, and carrots often have punctures, cuts, or splits that might be sites for bacterial

TABLE 11.4
Efficacy of H₂O₂-Based Washes for Decontamination
of Punctured Golden Delicious Apples Inoculated
with *E. Coli* (ATCC 25922)^a

Treatment ^b	Log ₁₀ CFU/g Reduction ^c	
	No Puncture	Punctured ^d
5% H ₂ O ₂	2.34	0.58
1% APL-Kleen® 245; 5% H ₂ O ₂ ^e	2.83	1.62

^a From Sapers, G.M. et al., *J. Food Sci.*, 65:529–532, 2000a.

^b 1-min wash at 50°C.

^c Means of duplicate trials; based on control populations of 4.88 log₁₀CFU/g.

^d 1-cm deep puncture made with 3.7-mm diameter sterile nail on top of apple 2 to 3 cm from stem.

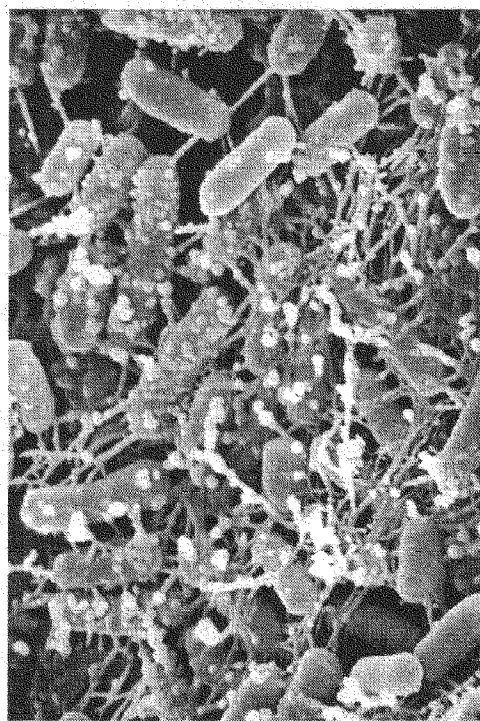
^e Two-stage treatment.

attachment. *E. coli* can grow within punctures in artificially inoculated apples in spite of the fact that this organism normally will not grow in highly acidic apple juice (Sapers et al., 2000a). Presumably, the bacteria are able to create a more hospitable microenvironment within the confines of the puncture. Other investigators have reported growth of *E. coli* in wounds on apples (Janisiewicz et al., 1999a, b). Once the bacteria have become established within a puncture, they are very difficult to kill (Table 11.4). A 5% hydrogen peroxide wash reduced the *E. coli* population on inoculated apples with punctures by only 0.6 log, compared to a 2.3-log reduction on inoculated apples without punctures (Sapers et al., 2000a).

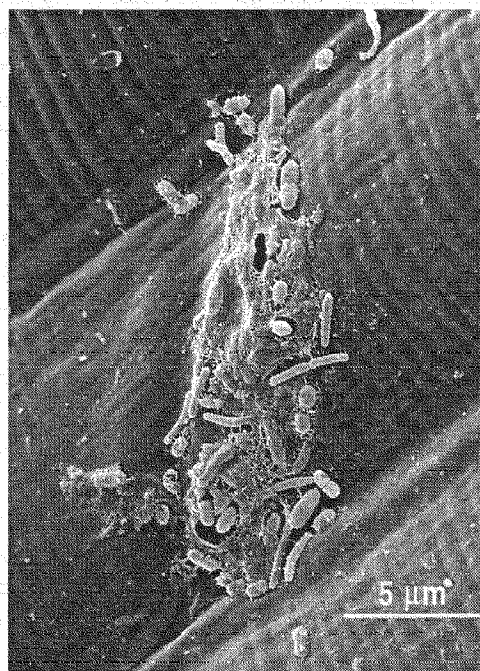
Biofilms

Attached bacteria might grow and form biofilms, bacterial communities adherent to a surface and each other by a self-produced polysaccharide matrix (Zottola, 1994; Costerton, 1995; Carmichael et al., 1999). Alternatively, introduced bacteria may become part of existing biofilms produced by the native microflora. In this state, bacteria are very resistant to detachment or inactivation by antimicrobial washes. Human pathogens such as *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes*, as well as other bacteria such as *Pseudomonas* and *Erwinia* spp., can form biofilms on inert surfaces (Somers et al., 1994). Biofilms on plant surfaces can comprise mixed populations of Gram-positive and Gram-negative bacteria, yeasts, and filamentous fungi (Morris et al., 1997).

Examples of typical biofilms found in the calyx of an apple and on the surface of an alfalfa sprout are shown in Figures 11.1a and 11.1b, respectively. The presence of biofilms on surfaces of fruits and vegetables greatly limits the ability to decontaminate them successfully (Carmichael et al., 1999; Fett, 2000). Similarly, biofilm formation on processing equipment complicates effective cleaning and sanitation (Zottola, 1994). Trisodium phosphate, applied as a 2 to 8% solution, has been shown



(a)



(b)

FIGURE 11.1 Micrographs of bacterial biofilms on produce: (a) apple calyx; (b) alfalfa sprouts.

to be effective against biofilm cells of *E. coli* O157:H7 and *Campylobacter jejuni* on stainless steel. Biofilm cells of *Salmonella typhimurium* and *L. monocytogenes* on stainless steel and Buna-N rubber were more resistant to trisodium phosphate treatments (Somers et al., 1994).

Internalization and Infiltration of Bacteria within Produce

If human pathogens can penetrate into the interior of a fruit or vegetable, they will survive surface decontamination treatments such as washing and surface pasteurization with hot water or steam. Internalization of bacteria can result from infiltration due to handling conditions during packing or processing (Bartz and Showalter, 1981; Bartz, 1982; Buchanan et al., 1999). Internalization also can occur naturally due to contamination of the flowering plant or during fruit development (Samish et al., 1963). It is not unusual to find a peach with a moldy pit or some other fruit or vegetable with a latent internal infection. Internalization of *E. coli* O157:H7 has been reported in lettuce (Seo and Frank, 1999; Takeuchi and Frank, 2000) and radish sprouts (Itoh et al., 1998), while other bacterial species have been detected within cucumbers and tomatoes (Samish et al., 1963; Meneley and Stanghellini, 1974; Daeschel and Fleming, 1981; Breidt et al., 2001).

Infiltration of bacteria into produce can occur in warm commodities with internal air spaces when they are placed in colder water, perhaps in a dump tank or flume. This can happen in the summer when the warm fruit or vegetable is brought in from the field and is washed with cool water obtained from a well. As the fruit or vegetable cools, the internal gas contracts, thereby creating a partial vacuum that will draw in water through pores, channels, or punctures. If the water contains human pathogens or microorganisms capable of causing spoilage, they also will be drawn into the commodity (Bartz and Showalter, 1981; Buchanan et al., 1999). Commercial experience has shown that tomato dump tank water temperature should be about 5°C higher than the fruit temperature to minimize infiltration (Hurst, 2001). Infiltration can occur when the commodity is at the bottom of a deep tank, but in this case, the driving force is hydrostatic pressure (Bartz, 1982; Sugar and Spotts, 1993). One can speculate that infiltration also might occur in the absence of an external driving force if the bacteria on the commodity surface are motile, i.e., they can move of their own accord, and they follow a channel into the interior of a fruit or vegetable when adequate surface water is available. In some apple cultivars, an open channel from the calyx leads directly to the core (Miller, 1959).

Infiltration of *Erwinia carotovora* subsp. *carotovora*, a spoilage organism, and *Salmonella montevideo*, a human pathogen, has been demonstrated in tomatoes (Bartz and Showalter, 1981; Zhuang et al., 1995). Infiltration of *E. coli* O157:H7 into Golden Delicious apples was demonstrated in the author's laboratory (Buchanan et al., 1999).

Microbial Interactions Favoring Pathogen Growth

When human pathogens contaminate a fruit or vegetable, they interact not only with the commodity surface but with the native microbial populations on the commodity, as well. In some cases, the interaction might be antagonistic, resulting in a decline

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in the pathogen population. In other cases, a synergistic or commensal relationship might favor pathogen survival or growth.

In studies carried out in the author's laboratory, Wells and Butterfield (1997) reported a strong statistical association between the occurrence of bacterial soft rot and the presence of presumptive *Salmonella*. In further testing, they found that about 30% of isolates from these samples were confirmed as *Salmonella*. It is not clear whether the association resulted from some positive interaction between *Salmonella* and the decay organisms or from the greater availability of nutrients in the macerated tissue that resulted from soft rot. Liao and Sapers (1999) reported an antagonistic relationship between soft-rotting bacteria on potato slices and *L. monocytogenes* strain Scott A. *Pseudomonas fluorescens* suppressed growth of *Listeria*, but *E. carotovora*, another soft-rotting species, permitted growth of *Listeria*. Thus, one cannot generalize about interactions between human pathogens and the endogenous microflora.

In a study of *E. coli* O157:H7 survival and growth in puncture wounds on apples, Riordan et al. (2000) reported that the human pathogen would die off quickly if the wound were infected with the common apple pathogen, *Penicillium expansum*. This was due in part to pH reduction caused by the fungus. However, if the wound were infected by *Glomerella cingulata*, another fungus associated with apple decay, the pH increased greatly, and *E. coli* O157:H7 grew and survived over a long period of time. *E. coli* O157:H7 grew equally well if the wound was not infected with a fungal pathogen; however, such growth was difficult to demonstrate because of unintended infection of inoculated wounds with native *Penicillium*. These results suggest a potentially hazardous situation in which apples punctured in a fall from a tree might be simultaneously infected with *E. coli* O157:H7 and *G. cingulata*. The decayed apple might eventually contain a population of *E. coli* O157:H7 sufficient to contaminate a large volume of apple cider.

These interactions have several important consequences. In the case of an antagonistic relationship, if a commodity is sanitized effectively so that the native antagonistic bacterial population is greatly reduced, a surviving human pathogen or newly introduced contaminant might get a foothold and grow without competition. The product might develop a dangerous level of the pathogen without ever showing visible spoilage. This was observed with vacuum-packed sliced potatoes artificially inoculated with *L. monocytogenes*, during storage at 15°C, an abusive condition (Juneja et al., 1998). That is why there is a concern about the development of washing treatments that extend product shelf-life by greatly reducing the population of spoilage organisms. One might be making the commodity "too clean." A second consequence is the possibility that a decayed fruit or vegetable might contain localized but not necessarily conspicuous areas where the population of a human pathogen is very high as a result of a synergistic or commensal interaction with the decay organism. This fruit or vegetable might be incorporated into a product such as unpasteurized cider if inspecting and sorting procedures are inadequate.

Detection and Removal of Produce with Defects

Fruits and vegetables with punctures, cuts, or decayed areas are at risk of being contaminated with human pathogens; thus, it would be desirable to cull defective

produce from packing or processing lines to reduce the risk of contamination. Inspection and sorting of incoming raw material are standard operations in packing and processing plants, but these operations are subject to human error, especially at high production rates, when inspectors are fatigued or when defects are inconspicuous. Small companies may not be set up properly for inspection and sorting or may lack the personnel to carry out these operations. Apples with decay spots and punctures have been observed in use for cider production because inspection was inadequate.

Technology for automated defect detection and sorting of produce is under development, but a number of technical problems must be solved before the equipment becomes available. This technology also may be too costly for the small packer or processor. Thus, produce packers and processors must ensure that they have adequate resources to inspect and sort raw material properly to exclude fruits or vegetables with potentially contaminated defects.

CONVENTIONAL WASHING TECHNOLOGY

EQUIPMENT AND MODES OF OPERATION

A number of types of commercial washers are used to wash fruits and vegetables (Figure 11.2). For commodities such as apples and potatoes, a brush washer should be used. Two types are available: flat-bed washers in which rotating brushes are arranged in a horizontal plane perpendicular to the flow of product, and U-bed washers in which the rotating brushes are arranged in a U-shaped configuration parallel to the flow of product. Brushes are tailored to the characteristics of specific commodities and are designed to scrub off adhering soil without damaging the product skin. Various other types of washers are available for leafy vegetables, broccoli, root vegetables, corn, etc. These include reel washers, pressure washers, hydro air agitation wash tanks, and immersion pipeline washers. (See Table 11.1 for sources.) While these units are designed to remove soil and pesticide residues, not much is known about their efficacy in removing bacterial contaminants.

To obtain such information, Sapers and colleagues conducted a series of washing trials with inoculated apples, using conventional and experimental wash solutions applied with commercial brush washers. Preliminary washing trials carried out with a U-bed brush washer indicated that population reductions were less than 1 log when apples inoculated with a nonpathogenic *E. coli* were washed with water, 200 ppm Cl_2 (as sodium hypochlorite), a commercial acidic detergent formulation, 8% trisodium phosphate, or 5% hydrogen peroxide applied at 20 or 50°C (Sapers and Jantschke, 1998). Under laboratory conditions in which the apples were submerged in the washing agents, some of these treatments had produced population reductions between 2 and 3 logs.

Further washing trials carried out with a commercial flat-bed brush washer confirmed these results (Annous et al., 2001). Population reductions for apples inoculated with a nonpathogenic *E. coli* were less than 1 log with the same washing agents used in the earlier trials (Table 11.5). The poor performance of both brush washers was attributed to deficiencies in equipment design resulting in insufficient exposure of inoculated apple surfaces to the washing agents, especially in the

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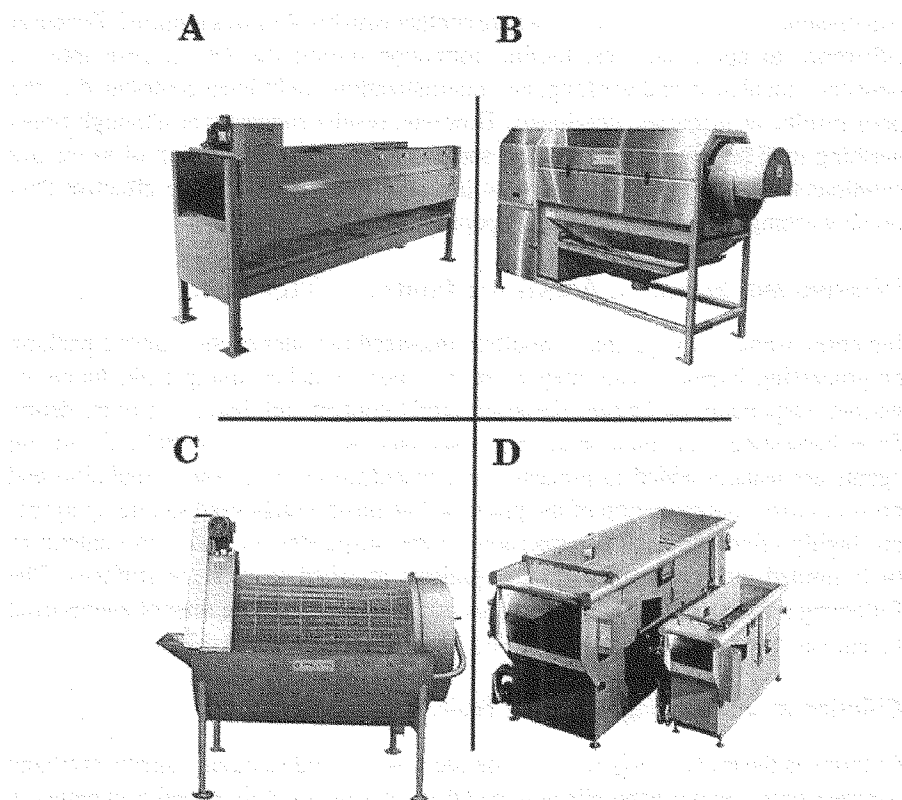


FIGURE 11.2 Commercial washing equipment for fruits and vegetables: (a) flat-bed brush washer; (b) U-bed brush washer; (c) rotary washer; (d) pressure washer.

TABLE 11.5
Decontamination of Apples Inoculated with *E. Coli* (Strain K12)
with Sanitizing Washes Applied in a Flat-Bed Brush Washer^a

Wash Treatment	Temperature (°C)	<i>E. coli</i> (log ₁₀ CFU/g) ^b		
		Before Dump Tank	After Dump Tank	After Brush Washer
Water	20	5.49 ± 0.09	4.92 ± 0.37	4.81 ± 0.26
	50	5.49 ± 0.09	5.03 ± 0.15	4.59 ± 0.08
200 ppm Cl ₂	20	5.87 ± 0.07	5.45 ± 0.05	5.64 ± 0.23
8% Na ₃ PO ₄	20	5.49 ± 0.09	5.02 ± 0.43	4.98 ± 0.02
	50	5.49 ± 0.09	5.02 ± 0.08	4.75 ± 0.45
1% acidic detergent	50	5.87 ± 0.07	5.49 ± 0.03	5.42 ± 0.50
5% H ₂ O ₂	20	5.87 ± 0.07	5.46 ± 0.40	5.27 ± 0.09
	50	5.87 ± 0.07	5.54 ± 0.31	5.49 ± 0.10

^a From Annous, B.A. et al., *J. Food Prot.*, 64:159–163, 2000.

^b Mean of 4 determinations ± standard deviation.

inaccessible calyx and stem areas, where contact with brushes was minimal. Bacterial adherence to apple surfaces, biofilm formation during the 18- to 24-h interval between inoculation and washing, and internalization might have contributed to the poor results, as discussed previously. However, results suggest that although brush washing may be needed for cleaning some commodities, application of sanitizing solutions to produce by full immersion in a dip tank would be more effective than brush washing in reducing microbial populations.

WASHING AND SANITIZING AGENTS FOR FRUITS AND VEGETABLES

Incoming fruits and vegetables are often immersed in water as they enter a packing or processing location. This may be in a cooler, drencher, dump tank, flume, or washer. Depending on its use, the water might contain soil, leaves, or other debris from harvesting and microorganisms associated with these materials. Sanitizing agents are usually added to process water to reduce the microbial population and prevent cross-contamination of the product. The most widely used sanitizing agents are highly effective in killing microorganisms suspended in water, in contrast to their limited efficacy against microorganisms attached to produce surfaces. The following sections look at the advantages and limitations of a number of agents used to sanitize fruits and vegetables (Table 11.6).

Chlorine as a Sanitizing Agent for Fruits and Vegetables

Chlorine is the most widely used among the washing and sanitizing agents available for fresh produce; it is generally assumed that chlorine is highly effective in reducing bacterial populations on commodity surfaces (Beuchat, 1998; Brackett, 1999). However, published data indicate that the most that can be expected at permitted concentrations is a 1- to 2-log population reduction (Brackett, 1987; Zhuang et al., 1995; Wei et al., 1995; Zhang and Farber, 1996; Beuchat et al., 1998; Sapers et al., 1999b; Pirovani et al., 2000). This is due in part to the inaccessibility of attached microorganisms and resistance of bacteria within biofilms but also to the rapid breakdown of chlorine in the presence of organic matter in soil and on product surfaces. Typically, chlorine is applied at a concentration no greater than 200 ppm, and solutions are adjusted to a pH of 6.5 to 7.5 to provide a high concentration of hypochlorous acid, the active form. Some improvement in the efficacy of chlorine can be obtained by addition of a wetting agent (Spotts and Cervantes, 1987). Washing formulations containing sodium hypochlorite, buffers, and surfactants are available commercially (Park et al., 1991; Wartanessian, 1997; Tenzer, 1997).

Another means of improving the efficacy of chlorine treatments is to monitor the oxidation-reduction potential (ORP) of the process water and to use this value as a means of controlling the treatment by addition of more hypochlorite or pH adjustment. An ORP value of 650 mv is recommended (Suslow et al., 2000). Commercial systems for controlling chlorine treatments, based on ORP measurements, are available (Vogel, 1999) (Table 11.1).

The use of electrolyzed water as a sanitizing agent for produce represents a special case of chlorination (Izumi, 1999). When water containing a small amount

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TABLE 11.6
Advantages and Disadvantages of Commercially Available Sanitizing Agents for Washing Fresh Fruits and Vegetables

Sanitizing Agent	Use Level (ppm)	Advantages	Disadvantages
Chlorine	50 to 200	Easy to apply Inexpensive Effective against all microbial forms Not affected by hard water Easy to monitor FDA approved	Decomposed by organic matter Reaction products may be hazardous Corrosive to metals Irritating to skin Activity pH-dependent Population reductions limited to <1 to 2 logs
Ozone	0.1 to 2.5	More potent antimicrobial than chlorine No chlorinated reaction products formed Economical to operate Self-affirmed GRAS, but FDA review possible Activity not pH-dependent	Requires on-site generation Requires good ventilation Phytotoxic at high concentrations Corrosive to metals Difficult to monitor Higher capital cost than chlorine No residual effect Population reductions limited to <1 to 2 logs
Chlorine dioxide	1 to 5	More potent than chlorine Activity not pH-dependent Fewer chlorinated reaction products formed than with Cl ₂ Effective against biofilms FDA approved Residual antimicrobial action Less corrosive than Cl ₂ or O ₃	Must be generated on-site Explosive at high concentrations Not permitted for cut fruits and vegetables Population reductions limited to <1 to 2 logs Generating systems expensive
Peroxyacetic acid	≤80	Broad spectrum antimicrobial action No pH control required Low reactivity with soil Effective against biofilms FDA approved No hazardous breakdown products No on-site generation required Monitoring not difficult Available at safe concentration	Population reduction limited to <1 to 2 logs Strong oxidant; concentrated solutions may be hazardous

of sodium chloride is subjected to electrolysis, hypochlorous acid is generated at a concentration of 10 to 100 ppm available chlorine. The solution can be highly acidic (pH <3.0) or alkaline (pH ≥11.0), depending on the configuration of the system. Electrolyzed water is sometimes referred to as functional water or high oxidation potential (HOP) water. An oxidation-reduction potential of 1000 to 1150 mv is desired (Kawamoto, 1999).

The results of electrolyzed water treatments have been mixed. In a comparison of acidic electrolyzed water and acidified chlorine treatments applied to inoculated lettuce leaves, Park and co-workers (2001) reported reductions of 2.49 and 2.22 log₁₀CFU/lettuce leaf for *E. coli* O157:H7 and *L. monocytogenes*, respectively. However, the difference between the two treatments was not significant. One study claimed a 3.7- to 4.6-log reduction of *E. coli* O157:H7 on apples treated with pH 2.6 electrolyzed water (Horton et al., 1999), but another study could demonstrate only a 1-log reduction in the microbial population on fresh-cut vegetables (Izumi, 1999). Such inconsistencies may be attributed to experimental differences in the interval between contamination and treatment, leading to differences in degree and strength of bacterial attachment and the opportunity for biofilm formation.

The reaction of chlorine with organic residues can result in the formation of potentially mutagenic or carcinogenic reaction products (Chang et al., 1988; Hidaka et al., 1992). This is a cause for concern because some restrictions in the use of chlorine might eventually be implemented by regulatory agencies.

Detergent Formulations and Other Commercial Produce Washes

Numerous commercial washing formulations have been developed for fruits and vegetables. (See Table 11.1 for sources.) They include various surfactants, combinations of surfactants with organic or mineral acids, and alkaline formulations based on sodium hydroxide. Relatively little information on their efficacy in reducing microbial populations on fruits and vegetables has been published. Wright et al. (2000) reported similar population reductions with a commercial phosphoric acid fruit wash and with a 200 ppm hypochlorite wash, each applied to apples inoculated with *E. coli* O157:H7. Sapers et al. (1999b) tested the efficacy of some commercial washing formulations in decontaminating apples artificially inoculated with non-pathogenic *E. coli* and found that these formulations were generally similar to chlorine, achieving a 1- to 2-log population reduction. When these products were applied at 50°C instead of at ambient temperature, a log reduction of about 2.5 could be obtained.

Ozone

Ozone is one of several new sanitizing agents for produce introduced in recent years (Graham, 1997; Xu, 1999). The efficacy of ozone in killing human pathogens and other microorganisms in water is well established (Wickramanayake, 1991; Restaino et al., 1995; Kim and Yousef, 2000), and it is widely used in bottled water purification systems as an alternative to chlorine (Graham, 1997). Ozone is effective in reducing bacterial populations in flume and wash water and may have some applications as

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a chlorine replacement in reducing microbial populations on produce (Achen and Yousef, 1999; Kim et al., 1999; Smilanick et al., 2000). Use levels of 0.5 to 4.0 µg/ml are recommended for wash water and 0.1 µg/ml for flume water (Zagory and Hurst, 1996; Strasser, 1998). However, ozone treatment (5.5 µg/ml water for 5 min) was ineffective in reducing postharvest fungal decay of pears (Spotts and Cervantes, 1992). Treatment of lettuce, inoculated with *P. fluorescens*, with ozone (10 µg/ml for 1 min) achieved less than a 1-log population reduction (Kim et al., 1999).

One of the major advantages claimed for ozone is the absence of potentially toxic reaction products. However, ozone must be adequately vented to avoid worker exposure and it must be generated on-site by passing air or oxygen through a corona discharge or UV light (Xu, 1999). A number of commercial systems for generating ozonated water for produce washing are available. (See Table 11.1 for sources of ozone generators.) An independent expert committee, sponsored by the Electric Power Research Institute, recommended that use of ozone as a disinfectant or sanitizer for foods be classified as “generally recognized as safe” (GRAS) (Graham, 1997). However, FDA may require further review to determine whether ozone treatments affect nutrient levels (Anonymous, 1999a).

Chlorine Dioxide

Chlorine dioxide also is used as an antimicrobial agent for produce washing. It is efficacious against many classes of microorganisms at lower concentrations than would be required with chlorine (Dychdala, 1991). It can reduce microbial populations in dump tank and wash water, but tests with cucumbers resulted in less than a 1-log population reduction on product surfaces (Costilow et al., 1984). In tests conducted in an apple packinghouse, addition of chlorine dioxide (3 to 5 µg/ml) to dump tank water reduced the population of filamentous fungi (Roberts and Reymond, 1994). Treatment of pears inoculated with *Botrytis cinerea*, *Mucor piriformis*, or *P. expansum* with 10 µg/ml chlorine dioxide for 10 min suppressed decay, but addition of 0.5 µg/ml of chlorine dioxide to flume water did not reduce decay of inoculated fruit (Spotts and Peters, 1980). A chlorine dioxide product, Oxine® (Bio-Cide International, Inc., Norman, OK), reduced the population of *E. coli* O157:H7 on inoculated apples by only 2.5 logs at 80 µg/ml, 16 times the recommended concentration (Wisniewsky et al., 2000).

Chlorine dioxide is approved for use on uncut produce (21CFR173.325, 2000). It produces fewer potentially carcinogenic chlorinated reaction products than chlorine (Tsai et al., 1995; Rittman, 1997); however, it is explosive and must be generated on site. A number of companies produce systems for in-plant generation of chlorine dioxide from stable precursors comprising sodium hypochlorite; hydrochloric, citric, or phosphoric acid; and sodium chlorite. (See Table 11.1 for sources of chlorine dioxide generators.)

Peroxyacetic Acid

Peroxyacetic acid, or peracetic acid as it is sometimes called, is actually an equilibrium mixture of the peroxy compound, hydrogen peroxide, and acetic acid (Ecolab,

1997, 2000). The superior antimicrobial properties of peroxyacetic acid are well known (Block, 1991). This agent is recommended for use in treating process water, but one of the major suppliers is also claiming substantial reductions in microbial populations on produce surfaces. However, the best that could be claimed with cut corn was a 1-log reduction (Ecolab, 1997). Population reductions for aerobic bacteria, coliforms, and yeasts and molds on fresh-cut celery, cabbage, and potatoes treated with 80 ppm peroxyacetic acid were less than 1.5 logs (Hilgren and Salverda, 2000). Several published studies have looked at the efficacy of peroxyacetic acid against *E. coli* O157:H7 on inoculated apples.

In one study in which apples were washed about 30 min after inoculation, the commercial peroxyacetic acid formulation (80 ppm peroxyacetic acid) reduced the *E. coli* population by about 2 logs, compared to a water wash (Wright et al., 2000). In another study, where the inoculated apples were held for 24 h before washing, the peroxyacetic acid treatment reduced the *E. coli* population by less than 1 log at the recommended concentration (80 ppm) and by 3 logs at 16 times the recommended concentration (Wisniewsky et al., 2000). Similar results with apples inoculated with a nonpathogenic *E. coli* have been reported (Sapers et al., 1999b). Like ozone and chlorine dioxide, peroxyacetic acid is effective in killing pathogenic bacteria in suspension at lower concentrations than would be required with chlorine (Block, 1991). Addition of octanoic acid to peroxyacetic acid solutions increased efficacy in killing yeasts and molds in fresh-cut vegetable process waters but had little effect on population reductions on fresh-cut vegetables (Hilgren and Salverda, 2000). Peroxyacetic acid is approved for addition to wash water (21CFR173.315, 2000). It decomposes into acetic acid, water, and oxygen, all harmless residuals. It is a strong oxidizing agent and can be hazardous to handle at high concentrations but not at strengths marketed to the produce industry.

NEW TECHNOLOGY FOR DECONTAMINATION OF PRODUCE

NOVEL WASHING AND SANITIZING AGENTS

Hydrogen Peroxide

The author has had extensive experience with hydrogen peroxide, which is also a strong oxidizing agent effective against a wide range of bacteria but less active against fungi (Block, 1991). Hydrogen peroxide vapor treatments have been used to inhibit postharvest decay in grapes (Forney et al., 1991), melons (Aharoni et al., 1994), and other commodities and to disinfect prunes (Simmons et al., 1997). However, vapor treatments tend to be slow and can cause injury to some commodities such as mushrooms, raspberries, and strawberries (Sapers and Simmons, 1998). Dilute hydrogen peroxide solutions are effective in washing mushrooms (McConnell, 1991; Sapers et al., 1994, 1999a, 2001a), controlling postharvest decay of vegetables (Fallik et al., 1994), extending the shelf-life of fresh-cut vegetables and melons (Sapers and Simmons, 1998), and decontaminating apples containing nonpathogenic *E. coli* (Sapers et al., 1999b; 2000a).

Washing and Sanitizing Raw Materials

Studies in this laboratory have shown that 5% hydrogen peroxide solutions, alone or combined with commercial surfactants, can achieve substantially higher log reductions for inoculated apples than 200 ppm chlorine. When applied at a temperature of 50 to 60°C, reductions as great as 3 to 4 log₁₀ CFU/g have been obtained (Sapers et al., 1999b). With cantaloupe melon, a 5% hydrogen peroxide wash applied at 50 or 60°C to the whole melon prior to rind removal was superior to chlorine in extending the shelf-life of fresh-cut melon cubes. Visual observations of spoilage were consistent with the microbiological data showing suppression of bacterial growth following the peroxide treatment, perhaps indicative of injury to spoilage-causing bacteria (Sapers et al., 2001b). The hydrogen peroxide wash was highly effective in inactivating nonpathogenic *E. coli* and *S. Stanley* on inoculated cantaloupe within 24 h of inoculation but only partially effective if the inoculated melons were stored for several days prior to washing (Ukuku and Sapers, 2000; Ukuku et al., 2001). These efficacy data and the apple results probably indicate attachment of *E. coli* and *S. Stanley* to inaccessible sites and biofilm formation.

Hydrogen peroxide is GRAS for some food applications (21CFR184.1366, 1994b) but has not yet been approved as an antimicrobial wash for produce. It produces no residue because it is broken down to water and oxygen by catalase, an enzyme found throughout the plant kingdom. However, hydrogen peroxide is phytotoxic to some commodities, causing browning in lettuce and bleaching of anthocyanins in mechanically damaged berries (Sapers and Simmons, 1998). It is hazardous in high concentrations and must be handled with care. Numerous suppliers can provide technical information about hydrogen peroxide applications (see Table 11.1 for sources), but prior FDA approval would be required for it to be used in washing fresh produce.

Trisodium Phosphate

Trisodium phosphate solutions are effective in decontamination of animal carcasses (Lillard, 1994; Dickson et al., 1994). This treatment is effective against *E. coli* and *Salmonella* and has been used experimentally as a wash to decontaminate tomatoes (Zhuang and Beuchat, 1996) and apples (Sapers et al., 1999b). It has been shown to be effective against bacteria in biofilms on stainless steel or buna-N rubber chips (Somers et al., 1994); however, 2% trisodium phosphate was ineffective in killing *L. monocytogene* on lettuce (Zhang and Farber, 1996). Trisodium phosphate is classified as GRAS (21CFR182.1778, 2000) when used in accordance with good manufacturing practice and is marketed by Rhodia Food (Rhodia, 1998) under the brand names AvGard™ and Assur-Rinse® for treatment of poultry and beef. Experimental applications for disinfection of fruits and vegetables have employed concentrations as high as 12% (Rhodia, 1998).

Organic Acids

Organic acids such as lactic and acetic acids are effective agents against bacteria (Foegeding and Busta, 1991). Lactic acid dips and sprays are used commercially to decontaminate carcasses containing *E. coli* O157:H7, *L. monocytogenes*, and *Sal-*

monella (Purac, 1997; Castillo et al., 2001). Microbial population reductions on treated beef retail cuts were less than 1 log, but outgrowth during storage was suppressed by a residual effect (Kotula and Thelappurath, 1994). Lactic acid rinses are being recommended for decontamination of fruits and vegetables. Total plate count reductions of 1 to 1.5 logs were reported on endive rinsed with 1.9% lactic acid for 1.5 min (Purac, 1997). Acetic acid has been tested as an antimicrobial agent for apples. In one study, a 5% acetic acid wash was reported to reduce the population of *E. coli* O157:H7 on inoculated apples by about 3 logs. However, these apples were inoculated only 30 min prior to treatment, probably providing insufficient time for strong bacterial attachment and possible biofilm formation (Wright et al., 2000). In another study, apples that had been inoculated with *E. coli* O157:H7 and air dried for 30 min were treated with 5% acetic acid at 55°C for as long as 25 min. Although the *E. coli* population was greatly reduced in the apple skin and stem areas, as many as 3 to 4 logs survived in the calyx tissue (Delaquis et al., 2000). It is not clear whether organic acid treatments would produce off-flavors in treated produce.

Other Experimental Washes

Peroxidase-generated iodine, which has been used to kill *Salmonella* on chicken breast skin (Bianchi et al., 1994), may be applicable to produce, but efficacy data are lacking. Dipping for 10 min in a saturated solution of calcinated calcium, an alkaline product obtained by ohmic heating of oyster shells, was reported to reduce bacterial populations in cucumbers and radish sprouts (Isshiki and Azuma, 1995). Copper and silver ions are known to exert antimicrobial activity (Hurst, 1991) and have been used to disinfect swimming pool water (Tew, 2000). Addition of 0.50 ppm copper and 0.035 to 0.05 ppm silver ions to water used in produce packing lines and dump tanks has been recommended (Tew, 2000), but the regulatory status of such treatments is not known. Grapefruit seed extract has been reported to be effective in inhibiting postharvest decay of fruits and vegetables (Cho et al., 1994), and grapefruit oil is used as a constituent of a produce wash intended for home use (Procter & Gamble, 2001). Allyl isothiocyanate (AITC), a food preservative derived from cruciferous vegetables (i.e., *Wasabi* horseradish, mustard) and marketed in Japan under the name Wasaouro®, may have application as an antimicrobial agent for minimally processed fruits and vegetables (Tokuoka and Isshiki, 1994; Delaquis and Mazza, 1995). AITC may be applied as a dip or in the vapor phase. This product is marketed in the U.S. by Midori Pharmacia Corp. (150 East 52 St., New York, NY).

NOVEL MEANS OF APPLYING SANITIZING AGENTS

Vacuum Infiltration

One means of improving contact between a sanitizing agent and bacteria attached in inaccessible sites on produce surfaces is by application of the treatment solution under vacuum. This procedure might be expected to remove gas barriers that block penetration of the sanitizing agent into pores, punctures, or sites like the apple calyx channel. By applying a 5% hydrogen peroxide solution to inoculated apples under vacuum, the author was able to obtain greater population reductions in the calyx

TABLE 11.7

Surviving Bacterial Populations in Excised Calyx and Stem Areas of Golden Delicious Apples Inoculated with *E. Coli* (ATCC 25922) and Decontaminated by Washing or Vacuum Infiltration with 5% H₂O₂, with or without Stem Removal^a

Experiment	Treatment	Stem Removal	Population Reduction ^b Log ₁₀ CFU/g	
			Stem Tissue ^c	Calyx Tissue ^c
A	5% H ₂ O ₂ at 60°C	No	2.96	2.40
		Yes	2.97	3.54
	5% H ₂ O ₂ vac. infilt. at 45°C	No	1.69	5.25
		Yes	3.07	5.16
B	5% H ₂ O ₂ at 60°C	No	3.30	2.76
		Yes	2.70	2.82
	5% H ₂ O ₂ vac. infilt. at 45°C	No	1.23	3.79
		Yes	3.34	4.81

^a Apples washed with 5% H₂O₂ at 60°C for 2 min or vacuum infiltrated with 5% H₂O₂ at 45°C and 100 mm Hg for 3 min; apples rinsed with H₂O after treatment.

^b Based on inoculated control (without stem removal) *E. coli* population of 5.29 and 6.38 log₁₀CFU/g in stem and calyx areas, respectively, for Expt. A and 5.09 and 6.53 log₁₀CFU/g in stem and calyx areas, respectively, for Expt. B.

^c Stem and calyx tissues excised aseptically from individual apples, weighed, and homogenized for enumeration of surviving *E. coli*.

area than were possible without the vacuum (Table 11.7). Treatment temperature and vacuum level were selected to minimize boiling of the hydrogen peroxide solution under vacuum, which might interfere with the treatment. Removal of stems prior to vacuum infiltration of hydrogen peroxide solution substantially improved treatment efficacy in the stem area. This may be due to the greater exposure of bacteria attached at the base of the stem to the sanitizing agent.

One of the limitations of this approach is the presence of sanitizer residue in the pores, channels, and punctures following infiltration treatment. This would not be an issue with hydrogen peroxide, which breaks down to oxygen and water soon after treatment. To carry out the vacuum infiltration treatment, processors would require a vacuum chamber, perhaps similar to that used for commercial vacuum infiltration of sugar, citric acid, and ascorbic acid into apple slices (Hall, 1989). This would be a batch process. Our observations indicate that vacuum infiltration of hydrogen peroxide is noninjurious to apples and might be applicable to apples intended for fresh market as well as for processing (Sapers et al., 2002).

Vapor-Phase Treatments

A vapor-phase antimicrobial treatment might be more effective than a wash in killing microbial contaminants attached in inaccessible sites. In studies carried out previ-

ously, the author and other investigators found that vapor-phase applications of hydrogen peroxide were effective in suppressing bacterial spoilage of mushrooms and other commodities but required exposure times as long as 60 min (Sapers and Simmons, 1998). With a more efficient means of vapor production to reduce the required treatment time, this use of hydrogen peroxide might have some application in disinfection of raw material for minimally processed fruits and vegetables.

Acetic acid vapor treatment of cabbage, mung bean seeds, and grapes has been reported to reduce microbial populations and prevent decay (Sholberg and Gaunce, 1995, 1996; Sholberg et al., 1996, 1998; Delaquis et al., 1997, 1999). A 5-log reduction has been obtained in the bacterial population of apples artificially contaminated with nonpathogenic *E. coli* and exposed to acetic acid vapor at 50°C (Sapers and Sites, 2001). The treated apples showed slight browning, which was indicative of injury and might limit use of this technology.

Vapor-phase disinfection of produce with chlorine dioxide (Han et al., 2000) has been investigated. Acetaldehyde has been reported to have antimicrobial properties and might have potential in inhibiting postharvest spoilage (Aharoni et al., 1973). It is not clear whether this treatment might be effective against human pathogens or would impart off-flavors to the treated commodities.

Surface Pasteurization

The surface of fresh fruits and vegetables might be pasteurized with hot water or steam, provided that required exposure times and temperatures were not capable of causing heat injury to the product, resulting in shortened shelf-life or altered flavor, color, or texture. Exposure of apples to water at temperatures above 65°C will cause the skin to brown, and exposure at 80°C for more than 30 sec will soften the outer 1 or 2 mm of flesh (Sapers et al., 2002). This would rule out treatments at temperatures greater than 65°C for fruit intended for fresh market. Such treatments would not be applicable to apples intended for cider production under newly promulgated FDA regulations (FDA, 2001). Fresh cantaloupes were found to tolerate exposure to water or 5% hydrogen peroxide at 80°C for 3 min with no adverse effects on appearance, flavor, or texture, initially or after storage at 4°C for 30 days (Sapers et al., 2000b).

A hot water washing system has been developed and commercialized in Israel for treatment of various fruits and vegetables (Fallik et al., 1996; Porat et al., 2000). This system is claimed to reduce the population of decay organisms and extend produce shelf-life, but data on inactivation of bacteria, especially human pathogens, are not available. A hot water decontamination treatment has been developed in the U.S. for cider apples (Dean, 1998). An experimental hot water pasteurization system developed by FDA scientists gave a 2-log reduction in apples inoculated with a nonpathogenic *E. coli* and then treated at 88 to 100°C (Keller, 1999). In other studies with apples inoculated with *E. coli* O157:H7 and surface pasteurized in water at 95°C, FDA scientists demonstrated a 5-log reduction when the apples were inoculated by applying droplets of inoculum to the fruit surface. However, only a 1-log reduction could be obtained when the apples were inoculated by immersion in the inoculum. Presumably, the surviving bacteria were attached in areas protected from exposure, perhaps by internalization during inoculation (Fleischman et al., 2001).

TABLE 11.8
Surface Pasteurization at 80°C of Golden Delicious Apples and
Cantaloupe Inoculated with *E. Coli* (ATCC 25922)

Commodity	Treatment	Log Reduction ^a	Appearance of Skin	Softening of Surface
Apples	1 min in H ₂ O	1.59 ± 0.60	Severe darkening	Slight
	1 min in 5% H ₂ O ₂	2.96 ± 0.07	Severe darkening	Slight
Cantaloupe	30 sec in 5% H ₂ O ₂	2.44 ± 0.04	Same as control	None
	1 min in 5% H ₂ O	3.05 ± 0.03	Same as control	None
	3 min in 5% H ₂ O ₂	4.37 ^b	Same as control	None
	5 min in 5% H ₂ O ₂	4.37 ^b	Same as control	None

^a Based on counts on *E. coli* Petrifilm. Mean of duplicate trials ± standard deviation; log reductions for apples and cantaloupe expressed as log₁₀CFU/g and log₁₀CFU/cm², respectively.

^b No survivors detected; log reduction = control population.

Several methods of surface pasteurization of citrus fruit prior to juicing have been investigated (Beasley, 1999). Spraying with water at 93 to 99°C for 60 sec was claimed to achieve a 5-log reduction in fruit inoculated with *E. coli*. Exposure to steam for 30 sec in a steam tunnel reduced the population by 3.7 logs.

Whether such treatments can kill bacteria attached within inaccessible sites such as the calyx or stem areas of apples or within punctures is not clear. In preliminary studies with inoculated apples, immersion for 3 min in 5% hydrogen peroxide at 80°C gave a 3-log reduction in the *E. coli* population (Sapers et al., 2002) (Table 11.8). The large number of surviving *E. coli* (about 2 log₁₀ CFU/g) indicates that such treatments cannot be considered effective surface pasteurization. On the other hand, immersion of cantaloupe inoculated with *E. coli* (ATCC 25922) in 5% hydrogen peroxide at 80°C for 3 min resulted in a 4.4-log reduction (no survivors detected), with no indication of treatment-induced quality defects (Table 11.8). Such a treatment should be capable of decontaminating the external surface of the melon so that the flesh does not become cross-contaminated when the rind is removed during fresh-cut processing (Sapers et al., 200b).

Synergistic Treatment Combinations

Although the efficacy of individual antimicrobial treatments might be limited to 1- to 2-log reductions in microbial populations, several treatments applied sequentially might result in substantial improvements in efficacy due to synergistic interactions between treatments. An acidified surfactant treatment, applied in a brush washer to maximize soil removal, might be followed by a hydrogen peroxide treatment, applied by immersing the commodity in a dip tank. Other examples of treatment combinations with the potential for synergism include an acidified surfactant wash treatment combined with surface pasteurization, vacuum infiltration of hydrogen peroxide, or ozone solution, or with vapor-phase application of a sanitizer vapor. Research on the efficacy of such combinations would establish whether synergism could be obtained.

CONCLUSIONS

Washing and sanitizing raw material for minimally processed fruit and vegetable products are key steps in assuring microbiological quality and safety. Conventional washing and sanitizing agents and equipment for their application generally cannot achieve reductions in microbial populations greater than 1 to 2 logs (90 to 99%). Such reductions may result in improvements in product quality and shelf-life extensions, but they cannot exclude the possibility of human pathogen survival and the associated risk of food-borne illness.

The efficacy of conventional washing and sanitizing agents is limited by bacterial adherence to produce surfaces, attachment in inaccessible sites, formation of resistant biofilms, and penetration within commodities. Furthermore, conventional washing equipment may not provide sufficient exposure of contaminated produce surfaces to washing and sanitizing agents. Incremental improvements can be made in sanitizer formulation and equipment design, but these are unlikely to increase treatment efficacy greatly.

New washing technology is needed to overcome these deficiencies. New treatments must be superior in efficacy, approved by regulatory agencies, safe to apply, compatible with existing industry practices, and affordable. A number of new approaches show promise. These include washing with more powerful antimicrobial agents such as hydrogen peroxide solutions, vacuum infiltration of sanitizers, vapor-phase application of sanitizers, surface pasteurization, and use of synergistic treatment combinations. Such innovations might not be capable of achieving the greater than 5-log reductions in pathogen populations possible with true pasteurization treatments, but they might bring about large improvements in the microbiological quality and safety of minimally processed fruits and vegetables.

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